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PRINCIPAL INVESTIGATOR: Susan L. Mooberry, Ph.D.

CONTRACTING ORGANIZATION: University of Texas Health Sciences Ctr.
San Antonio, TX 78245-0549

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Susan L. Mooberry, Ph.D.

Email: mooberry@uthscsa.edu

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14. ABSTRACT
For many prostate cancer patients currently available drugs do not stop the lethal progression of the disease. There is a critical need for more effective therapies for the treatment of late-stage, metastatic prostate cancer. We will test the hypothesis that new drugs and/or drug leads for the treatment for metastatic prostate cancer can be identified from the understudied plants of Texas. In this effort we will prioritize the most promising plant extracts and then prepare large-scale quantities of the plant extracts using supercritical fluid extraction techniques and use this material for bioassay-guided fractionation of the biologically active constituents using modern chromatography techniques. The chemical structures of isolated compounds will be determined using NMR spectroscopy. Detailed biological evaluations will be conducted on compounds isolated.

15. SUBJECT TERMS
Plant extracts, prostate cancer, chemotherapy

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Introduction:

For many prostate cancer patients currently available drugs do not stop the lethal progression of the disease. There is a critical need for more effective therapies for the treatment of late-stage, metastatic prostate cancer. In this project we will test the hypothesis that new drugs and/or drug leads for the treatment for metastatic prostate cancer can be identified from the understudied plants of Texas. We recently completed a library of 1,088 plant extracts. These extracts are derived from plants that thrive in the challenging environmental conditions of South Texas. They represent understudied plant species and they were collected and extracted using techniques to preserve their chemical diversity. The extracts were evaluated for activity against two prostate cancer cell lines and these results suggest that new compounds with potent and specific activities against prostate cancer can be identified from this plant collation. In this effort we will prioritize the most promising plant extracts for bioassay guided fractionation based on multiple criteria including potency and efficacy in multiple prostate cancer cell lines, selectivity for cancer cells, ability to circumvent multidrug resistance and supply of plant material and estimated yield of extract. Bioassay-guided fractionation will be used to isolate the active agents and structures will be elucidated by NMR. Basic mechanistic studies will be conducted and 3 compounds will be tested in a murine xenograft model of human prostate cancer.

Body:

The research accomplishments of this effort in the 4 months that the grant was active will be summarized under each specific aim.

Specific Aim 1: Prioritize the most promising 25 plant extracts according to selectivity for a panel of prostate cancer cells as compared to normal cells, the ability to circumvent multidrug resistance, potency, efficacy and supply of source material.

In the 4 months that this grant was active we conducted the primary screening of our plant extract library that contains 1,088 plant extracts. Each extract was evaluated for cytotoxic activity against PC3 and DU-145 prostate cancer cell lines. Based on these initial results, each of the extracts that caused at least 50% inhibition of proliferation at a concentration of 20 μg/ml was evaluated in detail using the sulforhodamine B assay and a full dose response curve was generated and the IC_{50} value (the concentration that causes 50% inhibition of proliferation) was calculated for each extract in each cell line. A total of 48 extracts were evaluated in secondary assays. Table 1 shows the results of these evaluations. Of the 48 extracts completed a46 yielded IC_{50} values less than 25 μg/ml in at least one of the cell lines with 44 having IC_{50} values total of has an IC_{50} value of 20 μg/ml or less, a value that is recommended by the NCI to be a gold standard for yielding compounds with sufficient potency to be considered as drug candidates. Additionally, most of the top 48 extracts have been evaluated against the NCI/ADR cells line that has a high expression of P-glycoprotein, a protein that contributes to drug resistance.

Detailed literature evaluations were conducted for all of the 48 extracts prioritized for cytotoxic activity against prostate cancer cells and as a result of these evaluations an initial list of 23 extracts of top priority were selected for follow-on studies. These results are presented in Tables 1 and 2.

Specific Aim 2: Prepare large scale quantities of the plant extracts using supercritical fluid extraction techniques.

Large scale plant collections were conducted for 14 of the top 20 prioritized extracts. We completed large scale extractions on 14 of the plants and small scale re-extractions on the remaining 6 plants. The details are presented in Table 2. Biological activity against prostate cancer cells was retained in each of the new extracts.

Specific Aim 3: Conduct detailed biological evaluations of compounds isolated in Specific Aim 2.

No progress has been made on this aim.

Key Research Accomplishments:

Prioritized 20 of the 25 plants extracts that we will isolate compounds from during the course of this grant

Conducted large scale collections of 14 of 20 prioritized plants

Extracted large scale quantities of plant extracts from 14 of the 20 top prioritized plants

Reportable Outcomes:

None

Conclusion:

We are poised to begin bioassay –guided fractionation on 14 of the top 20 prioritized plants.

References:

None

Appendices:

None

Supporting Data:

Table 1 and 2 (attached)

Table 1: Prioritization of extracts by bioassay and prior work

SLM	IC50: NCI µg/ml	IC50: PC-3 (µg/ml)	IC50: DU-145 (µg/ml)	Literature Summary	Priority
003L1	6.8	18.4± 5.4	7.3 ± 2.6	No prior literature found	1
009L1	>50	1.6 ± 0.6	1.9 ± 0.8	No prior literature found	1
012L1	1.6 ± 0.5	8.36 ± 2.5	16.3 ± 3.0	Genus cytotoxic leaf chem. on 5 cell lines + compound protects against induced cell-death + whole plant chem. inhibits DNA synthesis in HL-60 cells	0
017L1	13.9 ± 0.15	12.0 ± 4.3	13.9 ± 4.2	No prior literature found	1
036L2	ongoing	18.1 ± 5.6	34.6 ± 19.7	No prior literature found	1
071L2	7.2±0.76	19 ± 3.45	>50	Genus primarily cytotoxic root extracts & chemistry w/ unknown plant part having cytotoxic & antitumor saponins.	3
076L1	2.51±0.83	4.84	0.94	Genus leaf chem. inhibit angiotensin-converting enzyme activity? + cytotoxic *root* chem. + cytotoxic/antitumor *stem/bark* chem	0
082L2	>50	5.1 ± 0.5	10.3 ± 6.2	Genus selective cytotoxic extract + antitumor leaf extract	0
096L1	3.35±0.92	3.9	3.1	Genus cytotoxic leaf chem. on 4 cell lines	0
120L1	16.4	12.8 ± 0.3	19.1 ± 3.5	No prior literature found	1
121L1	4.5	3.3 ± 0.8	3.3 ± 0.4	Genus cytotoxic *root* chem.	2
125L1	7.0	2.1 ± 0.9	3.7 ± 1.3	Species cytotoxic leaf chem. in 3 cell lines + cytotoxic *root* extract	0
143L1	16.5	16.1 ± 8.1	26.1 ± 9.0	No prior literature found	1
147L1	10.8 ± 2.1	8.2 ± 3.4	12.0 ± 1.4	No prior literature found	1
171L2	6.0 ± 2.1	26.2 ± 10	50.2 ± 6.7	No prior literature found	1
176L1	3.1 ± 3.7	2.8 ± 0.3	4.2 ±1.5	No prior literature found	1
183L1	9.6 ± 0.01	15.8 ± 3.6	20.7 ± 3.6	Genus selective cytotoxic extract & chem. & cytotoxic leaf extract on 4 cell lines	0
184L1	6.21±2.4	3.8 ± 1.9	5.7 ± 3.0	Genus cytotoxic leaf chem. on 3 cell lines + *root & flower* cytotoxic extract	3
188L1	1.80 ± 0.75	6.0 ± 1.2	7.3 ± 1.0	No prior literature found	1
196L2	8.22 ± 3.1	7.2 ± 1.6	6.9 ± 1.5	Genus cytotoxic leaf chem. on 4 cell lines	0
217L1	>50	3.2 ± 0.5	3.7 ± 0.7	Genus chem. selectively cytotoxic + chem. from *bulbs* cyto. in 12 cell lines	0
237L2	14.6 ± 7.6	13.3 ± 6.2	25.4 ± 3.9	No prior literature found	1
244L1	5.2 ±1.8	3.9 ± 1.5	4.0 ± 0.7	Genus cytotoxic leaf oil on 3 cell lines + *root* extract cytotoxic & antitumor + stem extract cytotoxic	0

SLM	IC50: NCI ug/ml	IC50: PC-3 (ug/ml)	IC50: DU-145 (ug/ml)	Literature Summary	Priority
261L2	ongoing	8.3 ± 1.5	7.5 ±2.8	Genus cytotoxic *fruit* & *gall* chem.	1
269L1	7.19	5.7 ± 1.5	7.5 ± 2.8	No prior literature found	1
272L1	>50	17.3 ± 1.3	18.3 ± 5.9	Only cytotoxicity from 1970's	3
276L1	14.12	9.4 ± 2.2	11 ± 0.7	No prior literature found	1
282L2	0.85±0.26	1.48	13.29	Genus leaf extract cytotoxic + cytotoxic chem. against normal cells + cytotoxic in brine shrimp lethality test	0
284L1	No follow up	50.9 ±3.5	50.3 ±2.4	Genus cytotoxic root chem.	0
303 L1	No follow up	1.35	1.21	Genus cytotoxic & antitumor chem. (mix w/ other plant) + root cytotoxic chem.	0
305L1	No follow up	2.87	4.32		0
317L1	ongoing	17.8 ± 4.6	14.2 ± 2.4	Genus cytotoxic extract chem. likely only from wood	2
323L1	ongoing	22.5 ± 7.8	30.4 ± 0.2	No prior literature found	2
332L1	No follow up	13.7	18.2	Genus selective cytotoxic leaf chem.	0
333L1	No follow up	No follow up	No follow up		0
334L1	No follow up	4.0 ±0.8	3.4 ±0.6	Genus cytotoxic whole plant chem. in 4 cell lines & antitumor extract	0
335L1	No follow up	No follow up	1.6		0
339L2	No follow up	12.7	30.8 ± 12.9	One from China- ranunculin cytotoxic	0
340L1	ongoing	6.3 ±4.7	7.2 ± 3.6	No prior literature found	2
341L1	ongoing	0.39	0.37	No prior literature found	3
344L1	ongoing	4.0 ± 0.8	3.4 ± 0.6	No prior literature found	1
345L1	ongoing	11.8 ± 2.5	8.8 ± 1.0	No prior literature found	2
350L1	ongoing	7.7 ± 2.9	10.6 ± 4.3	No prior literature found	1
357L2	ongoing	15.9 ± 3.2	17.6 ± 4.0	No prior literature found	1
362L1	No follow up	0.81	0.87	Species cytotoxic leaf chem. in 3 cell lines	0
364L1	No follow up	5.6 ± 3.9	18.2 ± 1.4	Genus cytotoxic leaf chem. + embryotoxic *flower* extract	0
365L1	No follow up	2.2 ±1.6	1.7 0.7		0
366L2	ongoing	16.5 ±2.2	15.0 ± 5.3	No prior literature found	1

Table 2: Collection and extract status of Top 20 Plant Extracts

SLM	IC50 PC-3 (ug/ml)	IC50 DU-145 (ug/ml)	Status	Amt extr. (g)	Amt dry(g) on hand
003L1	18.4± 5.4	7.3 ± 2.6	mass re-ext.	2.12	870
009L1	1.6 ± 0.6	1.9 ± 0.8	mass re-ext.	2.10	477
017L1	13.3 ± 4.1	12.6 ± 3.3	mass re-ext.	4.00	730
036L2	18.1 ± 5.6	34.6 ± 19	mass re-ext.	1.67	476
120L1	12.8 ± 0.3	19.1 ± 3.5	mass re-ext.	1.68	559
143L1	8.9 ± 3.6	9.5 ± 2.3	mass re-ext.	1.89	745
147L1	8.2 ± 4.6	12.0 ± 1.4	mass re-ext.	2.21	626
171L2	26.2 ± 10.0	50.2 ± 6.7	mass re-ext.	1.03	620
176L1	2.8 ± 0.8	4.2 ± 1.5	mass re-ext.	2.95	687
188L1	6.0 ± 1.2	7.3 ± 1.0	mass re-ext.	5.80	1,748
237L2	13.3 ± 6.2	25.4 ± 3.9	mass re-ext.	3.96	1,369
261L2	8.3 ± 1.5	7.5 ± 2.8	mass re-ext.	4.8	941
269L1	5.7 ± 1.5	7.5 ± 2.8	mass re-ext.	1.74	603
276L1	11.9 ± 5.5	10.3 ± 3.5	mass re-ext.	0.72	633
323L1	22.5 ± 7.8	30.4 ± 0.2	small re-ext.	0.006	none
340L1	6.3 ± 4.7	7.2 ± 3.6	small re-ext.	0.07	39.09
344L1	4.0 ± 0.8	3.4 ± 0.6	mass re-ext.	30.1	37.7
350L1	7.7 ± 2.9	10.6 ± 4.3	small re-ext.	0.02	27.5
357L2	15.9 ± 3.2	17.6 ± 4.0	small re-ext.	0.03	56.8
366L2	16.5 ±2.2	15.0 ± 5.3	small re-ext.	0.05	8.7